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PREVENTION OF *HAEMOPHILUS INFLUENZAE* TYPE B INFECTIONS IN HIGH-RISK INFANTS TREATED WITH BACTERIAL POLYSACCHARIDE IMMUNE GLOBULIN

MATHURAM SANTOSHAM, M.D., RAYMOND REID, M.D., DONNA M. AMBROSINO, M.D., MARK C. WOLFF, PH.D., JANNE ALMEIDO-HILL, B.S., CLAUDETTE PRIEHS, B.S., KATHY M. ASPERY, R.N., STEVE GARRETT, R.PH., LARRY CROLL, R.PH., STEPHAN FOSTER, PHARM.D., GERALD BURGE, R.PH., PETER PAGE, M.D., BONNIE ZACHER, L.P.N., RICHARD MOXON, M.B., B.CHIR., F.R.C.P., AND GEORGE R. SIBER, M.D.

Abstract Apache Indian infants have a high frequency of *Haemophilus influenzae* type b (Hib) and pneumococcal infections. Forty percent of Hib infections in these infants occur before the age of six months, when active immunization may not be protective. To evaluate the efficacy of passive immunization with a human hyperimmune globulin (bacterial polysaccharide immune globulin [BPIG]) prepared from the plasma of immunized adult donors, we randomly assigned 703 infants in a double-blind fashion to receive 0.5 ml of BPIG per kilogram of body weight (n = 353) or 0.5 ml of saline (n = 350) intramuscularly at 2, 6, and 10 months of age.

Hib-antibody levels were significantly higher in BPIG recipients than in placebo recipients at 4, 6, and 10 months of age ($P<0.001$). During the first 90 days after BPIG or placebo injection, no Hib or pneumococcal infections were detected in the BPIG group, whereas seven Hib infections (six cases of bacteremia and one of meningitis) and four pneumococcal infections (bacteremia) were detected in the placebo group ($P = 0.007$ and 0.06, respectively). During the fourth month, one case of Hib meningitis and two cases of pneumococcal bacteremia developed in the BPIG group, whereas there were no Hib or pneumococcal infections in the placebo group.

We conclude that BPIG given at four-month intervals provided significant protection against serious Hib disease for three months, and that in high-risk infants it might be used alone, perhaps at three-month intervals, or together with active immunization. (N Engl J Med 1987; 317:923-9.)

HAEMOPHILUS influenzae type b (Hib) is an important cause of serious infections in infants and children.[1-3] In the United States the highest attack rates of meningitis and other bacteremic infections due to Hib have been documented among certain American Indian and Alaskan Eskimo populations.[4,5] In these high-risk groups, the majority of invasive Hib infections have occurred in children under one year of age, with up to 40 percent of cases occurring before six months of age.[4,5] Therefore, prevention of Hib infections in young infants is a high priority for these populations.

The currently licensed Hib vaccine consists of the purified capsular polysaccharide of Hib. Unfortunate-

From the Division of Geographic Medicine and Pediatric Infectious Diseases, Departments of International Health and Pediatrics, Johns Hopkins University School of Hygiene and Public Health and School of Medicine, Baltimore; the Department of Medicine, Francis Scott Key Medical Center, Baltimore; the Laboratory of Infectious Diseases, Dana–Farber Cancer Institute, Harvard Medical School, Boston; Biologic Laboratories of the Centers for Disease Control, Massachusetts Department of Public Health, Jamaica Plain, Mass.; American Red Cross Blood Services, Northeast Region, Dedham, Mass.; the Department of Paediatrics, John Radcliffe Hospital, University of Oxford, Oxford, England; and the U.S. Public Health Service hospitals, Whiteriver and San Carlos, Ariz. Address reprint requests to Dr. Santosham at Johns Hopkins University, Department of International Health, Division of Geographic Medicine, 615 N. Wolfe St., Baltimore, MD 21205.

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ly, this vaccine is not sufficiently immunogenic to be protective in infants less than 18 to 24 months of age.[6] Hib vaccines prepared by covalently coupling the Hib capsular polysaccharide with a protein carrier antigen are undergoing clinical evaluation.[7-11] Although these conjugate vaccines are more immunogenic than purified polysaccharides, serum antibody responses after a single dose in two-month-old infants have been inconsistent.[9-11]

An alternative approach for protecting high-risk infants is to administer an immune globulin containing high concentrations of antibody to the Hib capsular polysaccharide. Recently, a human hyperimmune globulin called bacterial polysaccharide immune globulin (BPIG) has been prepared from the pooled plasma of adult blood donors immunized with Hib, pneumococcal, and meningococcal capsular polysaccharide vaccines.[12] This globulin has been shown to prevent Hib bacteremia and meningitis in infant rats.[13]

This report describes a double-blind study of the safety and efficacy of BPIG in preventing bacteremic Hib and pneumococcal disease among Apache infants, who are known to be at high risk for these diseases.[4]

Methods

Patient Population

Newborn infants of mothers living on the White Mountain and San Carlos Apache Indian Reservations in Arizona were enrolled in the study after written informed consent was obtained from the mothers.

Ethical Clearance

The study protocol was approved by the Joint Committee on Clinical Investigation of Johns Hopkins University School of Medicine, the Indian Health Service, and the tribal councils of the White Mountain and San Carlos Apache tribes.

Enrollment

The study was active from June 1, 1983, to April 23, 1986, at the White Mountain Apache Reservation and from January 1, 1985, to April 23, 1986, at the San Carlos Reservation. During these periods, 628 (72 percent) of the 874 newborn infants at the White Mountain Apache Reservation and 134 (62 percent) of the 217 newborn infants at the San Carlos Reservation were enrolled in the study.

Randomization and Blinding

Infants were randomly assigned to the BPIG or placebo group in blocks of four, with a table of random numbers, just before their first routine childhood immunization. To ensure double blinding, the randomization code was maintained by the pharmacy staff of each hospital and was not available to the parents or guardians of the infants, the investigators, or the hospital staff. BPIG and placebo injections were given by the pharmacy staff while the parents and investigators were not present.

Study Protocol

Infants assigned to the treatment group received BPIG (0.5 ml per kilogram of body weight) at 2, 6, and 10 months of age. Infants assigned to the control group were given placebo (0.5 ml of normal saline) at the same ages. BPIG or placebo was given intramuscularly at two separate sites in the same thigh in equally divided volumes.

When the infants were two and six months of age, BPIG or placebo injection was accompanied by diphtheria, pertussis, and tetanus immunization, but in the other thigh.

Laboratory Investigations and Preparation of BPIG

At birth (cord blood) and at 2, 4, 6, 10, 15, and 18 months of age, 1 ml of blood was obtained for measurement of serum antibody to the Hib capsular polysaccharide.

Additional specimens were obtained to investigate acute illnesses. For infants with fever (temperature >39°C), blood was obtained for culture and serum was obtained to detect the presence of Hib antigen. For hospitalized infants with acute respiratory illnesses, blood and tracheal aspirates (obtained with a laryngoscope) were cultured to detect the presence of bacterial pathogens. In addition, serum and urine were obtained to detect Hib antigen. For infants with suspected sepsis or meningitis, blood, cerebrospinal fluid, and urine were obtained for bacterial culture and detection of Hib antigen.

BPIG was prepared and characterized as previously described.[12]

Antibody Assays

Antibody to the capsular polysaccharide of Hib was measured in a Farr assay as previously described.[14] The lower limit of sensitivity of the assay was approximately 0.05 μg per milliliter. For purposes of statistical analysis, values falling below this limit were assigned a value of 0.025 μg per milliliter.

The minimum protective level of passively administered IgG antibody to the Hib capsule was estimated to be 0.05 to 0.15 μg per milliliter. This estimate is based on trough antibody levels in patients with agammaglobulinemia receiving conventional immune globulin prophylaxis[15] and on direct measurement of minimum protective levels in the infant rat model of Hib bacteremia and meningitis.[13]

IgG, IgM, and IgA antibodies to the Hib capsular polysaccharide were measured with an enzyme-linked immunosorbent assay as previously described.[16,17]

Microbiologic Studies

Samples of blood, cerebrospinal fluid, and other body fluids and tracheal aspirates were processed according to standard bacteriologic techniques.[18,19]

H. influenzae organisms were identified by tests of their dependence on X and V factors for growth[18] and were serotyped by agglutination with antiserums specific for type a or b antibodies (Difco Laboratories, Detroit). Organisms agglutinating only with type b antiserum were identified as Hib.

Blood, urine, and cerebrospinal fluid were tested for the presence of Hib antigen with use of the Wampole (Bactogen) latex-agglutination test. Antigen tests were confirmed with the Farr inhibition assay.[14]

Definitions of Major Outcomes

All infants who received at least a single dose of BPIG or placebo were included in the analysis. For assessment of the efficacy of BPIG, only outcomes occurring within four months of receipt of BPIG or saline were evaluated, on the basis of previous pharmacologic studies.[17] The major study outcomes were invasive Hib, pneumococcal, or meningococcal infections, defined as isolation of one of these organisms from a normally sterile body fluid (blood, cerebrospinal fluid, pleural fluid, or joint fluid), or the presence of purulent meningitis (>100 polymorphonuclear cells per cubic millimeter of cerebrospinal fluid) together with the presence of Hib capsular polysaccharide antigen in the cerebrospinal fluid. If an infant had more than one episode of invasive Hib or pneumococcal disease, only the first episode of illness was used for comparison of major outcomes between the groups.

Definitions of Minor Outcomes

Pneumonia was defined as the presence of clinical symptoms and signs of lower respiratory tract infection, as judged by the attending physician, together with roentgenographic abnormalities, as judged by the attending radiologist. Films initially interpreted as showing pneumonia were reviewed for consistency at the end of the study, by

a radiologist who did not know the treatment code. Lobar consolidation was defined as the presence of a homogeneous, dense infiltrate involving one or more segments or lobes.

Monitoring of Adverse Reactions to BPIG

Infants were observed for immediate reactions for 30 minutes after BPIG or placebo administration. Active monitoring of adverse effects was conducted by home visits 24 and 48 hours after injection of the 2-month dose in 113 subjects and after the 10-month dose in 100 subjects.

Decision for Stopping the Study

An independent operations committee advised the investigators when to stop the study. The committee was informed periodically of the number of invasive Hib and pneumococcal infections that had occurred within four months of BPIG or placebo administration. On April 23, 1986, when 14 major study outcomes had occurred (8 Hib and 6 pneumococcal infections), the committee elected to break the code. Before breaking the code, the committee decided on its recommendation to continue or stop the trial according to each possible distribution of Hib illnesses between the treated and placebo groups. After breaking the code, the committee advised stopping the trial because significant differences in the incidence of Hib infections had occurred.

Statistical Analysis

Unless otherwise indicated, two-tailed Fisher's exact test was used for comparison of the frequency of major and minor outcomes.

Comparisons of antibody concentrations in BPIG and saline recipients were performed on geometric means, using the t-test when they were normally distributed (according to the Wilk–Shapiro test) and the Wilcoxon nonparametric test when they were not normally distributed. The apparent half-lives of serum antibodies were calculated with use of values in the measurable range by methods previously described.[20]

Results

Clinical Characteristics

From June 1, 1983, to April 23, 1986, 762 newborn infants were enrolled in the study. Seven hundred three (92 percent) of these infants were randomly assigned to BPIG or placebo and received at least one injection. The remaining 59 infants (8 percent) were withdrawn from the study before receiving the first BPIG or placebo injection for one of the following reasons: the infant died (n = 3), the family moved from the reservation (n = 9), the mother requested that the infant be removed from the study (n = 17), or the infant was more than one month past due for the first immunization (n = 30).

Of the 703 infants who received the first BPIG or placebo injection, 353 were assigned to the treatment group and 350 to the placebo group. Selected characteristics of the infants are shown in Table 1. None of the differences were significant.

Table 1. Selected Characteristics of Infants Enrolled in the BPIG and Placebo Groups.*

	BPIG	Placebo
No. who received 1 dose	353	350
No. eligible to receive 2 doses†	312	305
No. who received 2 doses (%)	283 (91)‡	270 (89)‡
No. eligible to receive 3 doses†	232	227
No. who received 3 doses (%)	222 (96)‡	218 (96)‡
Total no. of doses	858	838
No. monitored until 18 months of age (%)	134 (38)	130 (37)
Days at risk for disease within 120 days of treatment (mean ±SE)	267.4±6.4	265.9±6.4
Person-years at risk for disease	259	255
M/F	162/191	167/183
Geographic distribution of infants — no. (%)		
Whiteriver	240 (68)	242 (69)
Cibecue	54 (15)	49 (14)
San Carlos	59 (17)	59 (17)
No. with birth weights ≤2500 g (%)	23 (7)	19 (5)

*None of the differences were statistically significant.
†Old enough at termination of the trial to receive the corresponding dose.
‡Percent of those eligible to receive the corresponding dose.

Antibody to Hib-CP in BPIG and Placebo Recipients

Figure 1 and Table 2 summarize the concentrations of antibody to the Hib capsular polysaccharide in 30 BPIG and 29 placebo recipients. During each of the first 15 months of the study, the first two infants enrolled in each arm of the study who met all of the following criteria were selected for this analysis: no documented Hib or pneumococcal infection, no detectable colonization with Hib or cross-reactive organisms, and the availability of relatively complete sets of serum samples.

At birth, maternal antibody to the Hib capsular polysaccharide was detectable at concentrations above 0.15 μg per milliliter in 94 percent of the placebo recipients and 90 percent of the BPIG recipients. Maternal antibody levels declined with a mean half-life of 32 days (range, 20 to 46). The mean half-life of BPIG-induced Hib antibody, calculated from the decline in serum antibody levels between four and six months in BPIG recipients, was 31.2 days (range, 20 to 66).

At six months of age, 22 of 29 infants (76 percent) receiving placebo had no detectable antibody to the Hib capsular polysaccharide (<0.05 μg per milliliter). In contrast, 27 of the 30 BPIG recipients (90 percent) still had detectable antibody concentrations at six months.

The acquisition of "natural" antibody by 18 months appeared to be slower in BPIG than in placebo recipients. Mean antibody concentrations (Fig. 1) and the proportion of children with undetectable antibody concentrations (Table 2) did not differ significantly at 15 and 18 months. However, the proportion of children with "natural" antibody levels above 0.15 μg per milliliter was significantly lower in BPIG recipients than in placebo recipients at 15 and 18 months (Table 2).

Major Outcomes

Between June 1, 1983, and April 23, 1986, 34 invasive infections due to Hib and pneumococci were observed in the study population; 2 occurred before vaccination. No meningococcal infections were detected. Table 3 summarizes the distribution of these infections. During the first three months after vaccination, there were seven Hib infections in the placebo group and none in the BPIG group (P = 0.007). During the

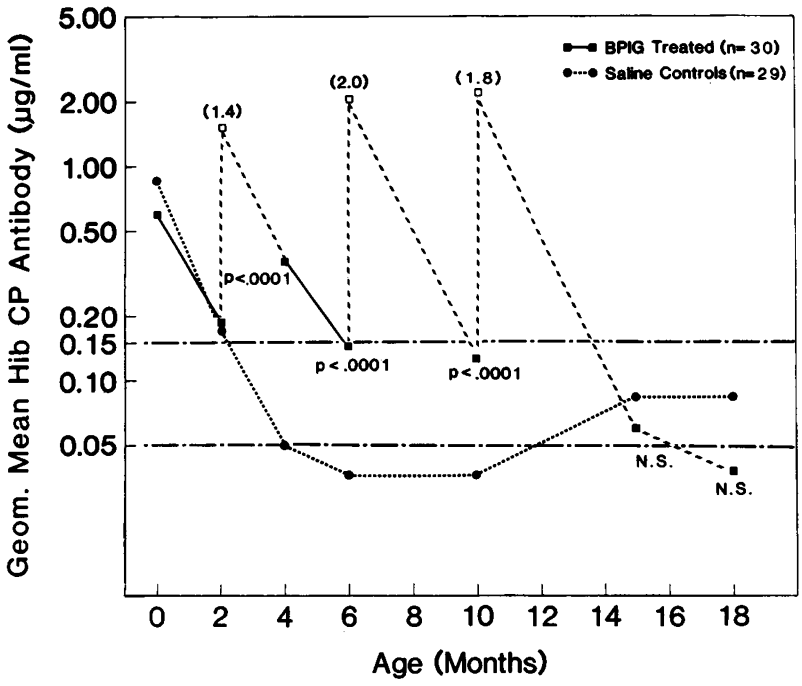

Figure 1. Mean Concentration of Hib Capsular Polysaccharide (Hib CP) Antibody in BPIG and Placebo Recipients.

The interrupted horizontal lines represent estimates of the minimum protective concentration of IgG class Hib antibody. In BPIG recipients, peak antibody concentrations (open squares) were extrapolated from observed values (closed squares), assuming a half-life of 30 days for the antibody. The extrapolated points are connected to observed values by interrupted lines. P values represent the significance of differences between the means according to the t-statistic.

same period, there were four pneumococcal infections in the placebo group and none in the BPIG group ($P = 0.06$). With both outcomes combined, there were 11 infections in the placebo group and none in the BPIG group ($P = 0.001$). During the fourth month, one Hib infection and two pneumococcal infections occurred in the BPIG group and none occurred in the placebo group. The difference in Hib infections in the two groups during the full four months remained significant ($P = 0.04$), as did the differences in Hib combined with pneumococcal infections ($P = 0.03$).

On the basis of the above data, the efficacy of BPIG in preventing Hib infection during the first three months after vaccination was 100 percent, with a 95 percent one-sided confidence interval of 46.7 to 100 percent. For the full four months, the efficacy was 85.8 percent, with a 95 percent confidence interval of −11.4 to 99.7 percent, according to the methods described by Ederer and Mantel.[21] During the subsequent two-year follow-up period, there were 6 Hib infections (2 in the BPIG group and 4 in the placebo group) and 12 pneumococcal infections (6 in each group).

We also monitored the incidence of Hib infections among infants in Whiteriver who were not enrolled in the study. During the study period, the population had a total of 786 person-years at risk — 279 (35.5 percent) for the BPIG group, 275 (35.0 percent) for the placebo group, and 232 (29.5 percent) for unenrolled infants. Of the 14 cases of Hib infection that occurred, 1 (7.1 percent) was in the BPIG group, 7 (50 percent) were in the placebo group, and 6 (42.9 percent) were in the unenrolled group. Therefore, the incidence density (cases per person-year) was significantly lower ($P = 0.027$) in the BPIG group than in the combined placebo and unenrolled groups.[22]

Six of the seven infants in the placebo group who had invasive Hib infections had positive blood cultures. Three of these infants had pneumonia, one had septic arthritis, one had meningitis with a positive cerebrospinal fluid culture, and one had bacteremia without a focus of infection. Another infant, three months of age, was given a diagnosis of meningitis in the absence of positive blood and cerebrospinal fluid cultures. This infant had received two doses of amoxicillin within 12 hours before hospitalization, and she had 9800 polymorphonuclear cells in her cerebrospinal fluid. Blood, urine, and cerebrospinal fluid specimens were all strongly positive for Hib antigen by latex agglutination and radioimmunoassay. Two weeks before her hospitalization, she had Hib in her oropharynx.

In one five-month-old infant, Hib meningitis developed 98 days after treatment with BPIG. On a routine visit two weeks before the illness, the child was found to be colonized with Hib and to have 0.32 µg of Hib capsular antibody per milliliter on the Farr assay. On admission, cultures of blood and cerebrospinal fluid yielded Hib, but an oropharyngeal swab obtained several hours after initiation of antimicrobial therapy revealed no Hib. A serum specimen obtained in the acute phase was strongly positive for Hib antigen by latex agglutination and contained 0.11 µg of Hib capsular antibody per milliliter (although a prozone effect was noted when undiluted serum was assayed). An enzyme-linked immunosorbent assay showed that most of the antibody present two weeks before admission and on admission was of the IgG class and was thus presumably passively acquired from BPIG. The child had only 0.01 µg of IgA Hib capsular antibody per milliliter in each of these two specimens. A serum sample obtained in the convalescent phase contained 0.46 µg of the Hib antibody per milliliter on the Farr assay, and most of the antibody was of the IgM class on enzyme-linked immunosorbent assay.

Of the four infants with pneumococcal bacteremia

Table 2. Proportion of Placebo and BPIG Recipients with Serum Concentrations of Hib Capsular Antibody below Estimated Protective Levels.*

	Age (Months)						
	0	2	4	6	10	15	18
Placebo recipients							
No. examined	18	26	29	29	29	28	28
Percent with <0.15 μg/ml	6	46	83	97	90	71	64
Percent with <0.05 μg/ml	0	19	55	76	86	54	54
BPIG recipients							
No. examined	20	24	30	30	28	30	30
Percent with <0.15 μg/ml	10	42	13†	50†	57†	93‡	93†
Percent with <0.05 μg/ml	5	25	3†	10†	14†	37	67

*The minimum protective level of passively acquired IgG Hib capsular antibody is estimated to be 0.05 to 0.15 μg per milliliter (see text).

†Significantly different from value in placebo recipients (P<0.01 by two-sided Fisher's exact test).

‡Significantly different from value in placebo recipients (P<0.05 by two-sided Fisher's exact test).

in the placebo group, two were given a diagnosis of otitis media and pneumonia, one had otitis media, and one had no focus of infection. Three of the infants had serotype 4 pneumococcus, and one had serotype 18. The two cases of pneumococcal infection in the BPIG group occurred 105 and 121 days after BPIG administration. One infant was given a diagnosis of pneumonia, and the other did not have an identifiable focus of infection. The pneumococcus from one of these patients was serotype 4, and that from the other patient was not serotyped.

Minor Outcomes (Table 4)

There were no statistically significant differences between the groups in the number of infants with pneumonia or the number hospitalized because of any illness after the BPIG or placebo injections. However, fewer infants in the BPIG group than in the placebo group had three or more episodes of pneumonia ($P = 0.007$). The frequency of consolidative pneumonia was also lower in the BPIG group ($P = 0.09$). Otitis media was not used as an outcome because the diagnosis rested solely on the clinical impressions of different physicians.

Adverse Effects

No immediate adverse reactions to BPIG or placebo immunizations were noted by the pharmacist. In addition, no infants presented to the hospital because of adverse effects attributed to BPIG or placebo.

Active follow-up at home for local reactions at two months of age revealed that 2 of 49 BPIG recipients (4 percent) had local swelling, erythema, and tenderness at the site of injection 24 hours after immunization. These reactions resolved within 48 hours of immunization. None of the placebo recipients had local adverse reactions. No local reactions were noted in either group after the 10-month immunization.

Discussion

We have demonstrated that passive immunization with a human hyperimmune globulin enriched in antibodies to Hib and pneumococcal capsular polysaccharides significantly reduced bacteremic infections caused by these organisms in a high-risk Apache Indian population. The reduction was statistically significant when Hib infections were analyzed separately and showed a trend with pneumococcal infections. Between 91 and 120 days after BPIG administration, one case of invasive Hib disease and two cases of invasive pneumococcal disease occurred in the BPIG group. In addition, direct antibody measurements indicated that 10 to 15 percent of infants no longer had detectable antibody to the Hib capsular polysaccharide four months after BPIG administration (Table 2). Administration of this globulin at three-month intervals may therefore be needed to achieve uninterrupted protection.

Since the code was broken in April 1986, BPIG given on a more frequent schedule (at birth and at two, four, six, and nine months of age) has been offered to all infants on the White Mountain and San Carlos Apache reservations. Four hundred ninety-seven infants have been immunized, and no invasive Hib infections have been observed among them over a 10-month period. In contrast, four cases of Hib infection have occurred among 343 other infants during this period.

Between the ages of 2 and 14 months, only one BPIG recipient in the study group had a bacteremic Hib infection. This child was first found to be colonized with Hib two weeks before meningitis developed. At that time the IgG antibody level was above the level considered protective. Nevertheless, Hib bacteremia and meningitis developed. On admission, the child had antigenemia and an undiluted serum speci-

Table 3. Comparison of BPIG and Placebo in Prevention of Hib and Pneumococcal Bacteremia and Meningitis.

	BPIG Group	Placebo Group	P Value*
No. of patients at risk	353	350	
No. of doses	858	838	
Cases of invasive Hib disease			
Before treatment period	0	1	
1–90 days after immunization	0	7	0.007
91–120 days after immunization	1	0	
1–120 days after immunization	1	7	0.04
After treatment period†	2	4	
Cases of invasive pneumococcal infections			
Before treatment period	1	0	
1–90 days after immunization	0	4	0.06
91–120 days after immunization	2	0	
1–120 days after immunization	2	4	
After treatment period†	6	6	
Combined no. of cases of invasive Hib and pneumococcal infections			
Before treatment period	1	1	
1–90 days after immunization	0	11	<0.001
91–120 days after immunization	3	0	
1–120 days after immunization	3	11	0.03
After treatment period†	8	10	
Total number of cases	12	22	

*By two-sided Fisher's exact test. P value is given only if P<0.1.

†At 121 days to two years after the third dose.

Table 4. Comparison of BPIG and Placebo in Prevention of Various Outcomes.*

	BPIG Group	Placebo Group	P Value†
No. of children at risk	353	350	
No. of episodes of pneumonia	29	35	
No. of children with ≥3 episodes of pneumonia	0	7	0.007‡
No. of children with consolidative pneumonia	3	9	0.09‡
No. of episodes of gastroenteritis	620	581	
No. of episodes of impetigo	24	30	
No. of children hospitalized with any illness	154	164	
No. of children hospitalized with temperature ≥39°C	100	123	0.05§

*At 1 to 120 days after treatment.
†P value is given only if P<0.1.
‡By two-sided Fisher's exact test.
§By chi-square test.

men did not show antibody activity on an assay of radioactive-antigen binding. However, upon dilution, antigen-binding activity became detectable. This observation suggests that an excess of antigen had formed a complex with the antibody in the undiluted serum.

The reason for the failure of passive immunization in this infant is not clear. One possibility is that Hib capsular polysaccharide antigen from the colonizing organism may have been absorbed and bound to the antibody. Formal investigations of the infant's immune functions were not done, but the previous and subsequent clinical courses were not marked by recurrent infections.

The active antibody response to tetanus and diphtheria toxoids given concurrently was not suppressed.[23] Also, the frequency of acquiring detectable "natural" antibody to the Hib capsular polysaccharide, presumably in response to unrecognized exposure to Hib or cross-reactive antigens, was similar in BPIG and placebo recipients. However, the frequency of relatively high concentrations of the "natural" antibody (>0.15 μg per milliliter) was significantly reduced (P<0.01, Table 2) in BPIG recipients. The reason for this reduction is not clear, but it may reflect a lower frequency of subclinical or unrecognized Hib infections or an impaired response to subclinical infection or colonization. However, in preliminary studies, the capacity to respond to Hib capsular polysaccharide vaccine at 18 months of age was not impaired in BPIG recipients relative to placebo recipients (Santosham M, et al.: unpublished data).

The role of BPIG in the prevention of encapsulated bacterial infections needs to be defined. The efficacy of BPIG in preventing Hib bacteremia and meningitis suggests that it should be used prophylactically in persons known to be at high risk for Hib infections, such as American Indians and Alaskan Eskimo infants.[4,5] In these populations, a substantial proportion of Hib infections occur in infants less than six months of age. The more immunogenic Hib vaccines currently being investigated do not reliably produce an antibody response at two months of age,[9-11] particularly in American Indians.[11] Since BPIG does not appear to suppress the active response to Hib capsular polysaccharide conjugate vaccine,[11] combined passive and active immunization could be used to provide uninterrupted protection to high-risk groups once both BPIG and the new conjugate vaccines are licensed.

Another use for BPIG would be as prophylaxis for exposed household contacts of patients with primary cases of Hib infection.[24] Currently, rifampin prophylaxis is recommended in this situation.[25] Unlike rifampin prophylaxis, BPIG prophylaxis could be restricted to children at risk and would not have to be given to all household contacts.

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MUTATIONAL ACTIVATION OF THE K-*RAS* ONCOGENE

A Possible Pathogenetic Factor in Adenocarcinoma of the Lung

SJOERD RODENHUIS, M.D., MARCUS L. VAN DE WETERING, B.S., WOLTER J. MOOI, M.D., SIEGINA G. EVERS, B.S., NICO VAN ZANDWIJK, M.D., AND JOHANNES L. BOS, PH.D.

Abstract To define the role of cellular oncogenes in human cancers, we studied the prevalence of mutational activation of *ras* oncogenes in untreated non-small-cell lung cancer. Genomic DNA was extracted from 39 tumor specimens obtained by thoracotomy and was examined for activating point mutations in codons 12, 13, and 61 of the H-*ras*, K-*ras*, and N-*ras* genes. A novel, highly sensitive assay based on oligonucleotide hybridization following an in vitro amplification step was employed.

The K-*ras* gene was found to be activated by point mutations in codon 12 in 5 of 10 adenocarcinomas. Two of these tumors were less than 2 cm in size and had not metastasized. No *ras* gene mutations were observed in 15 squamous-cell carcinomas, 10 large-cell carcinomas, 1 carcinoid, 2 metastatic adenocarcinomas from primary tumors outside the lung, and 1 small-cell carcinoma. An approximately 20-fold amplification of the unmutated K-*ras* gene was observed in a tumor that proved to be a solitary lung metastasis of a rectal carcinoma.

We conclude that mutational K-*ras* activation may be an important early event in the pathogenesis of adenocarcinoma of the lung but that amplification of *ras* genes or mutational activation of H-*ras* or N-*ras* does not play a major part in non-small-cell lung cancer. (N Engl J Med 1987; 317:929-35.)

DESPITE recent advances in our understanding of the molecular mechanisms that give rise to malignant behavior of cells, the role of known oncogenes in human cancer remains largely conjectural.[1] Oncogenes are derived from normal genes ("proto-oncogenes") that are highly conserved in evolution and that code for proteins having important roles in normal cellular processes, such as the regulation of gene expression or growth-signal transduction. Certain events may lead to structural abnormalities in or around these genes that make them contribute to the process of malignant transformation ("activation"). The most frequent mechanisms of this activation of proto-oncogenes in human cancers are (1) structural abnormalities of chromosomes associated with gene translocation, such as the Philadelphia chromosome in chronic myelocytic leukemia, with translocation of the *abl* gene[2]; (2) an increase in the number of copies of a particular gene per cell, termed "gene amplification"[3]; and (3) point mutation, in which a single base substitution in the DNA leads to a single amino acid substitution in the encoded protein, which then acquires transforming potential.

The oncogenes that appear to have a role in human lung cancer belong to two families of closely related genes, called *myc* and *ras*. The *myc* genes code for nuclear proteins that are believed to be involved in growth regulation. Amplifications of at least three *myc* genes (c-*myc*, N-*myc*, and L-*myc*) have been shown to occur in small-cell carcinoma cell lines; the overexpression of one of these, c-*myc*, appears to induce a different and even more malignant ("variant") phenotype.[4,5] *Myc* amplifications have also been noted in fresh tumor material from patients with small-cell carcinoma[6] and have been associated with tumor progression and an unfavorable prognosis.

Activated *ras* oncogenes have mainly been encountered in lung cancer cell lines of non-small-cell origin.[7-9] The genes of this family, of which H-*ras*, K-*ras*, and N-*ras* are well characterized, code for 21-kilodalton (kd) guanosine triphosphate–binding proteins (p21), which are related to G-proteins and are thought to play a part in growth-signal transduction.[10] The *ras*

From the Divisions of Experimental Therapy and Clinical Oncology, The Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Amsterdam; and the Department of Medical Biochemistry, State University of Leiden, Sylvius Laboratories, Leiden, The Netherlands. Address reprint requests to Dr. Rodenhuis, at the Department of Medical Oncology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

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